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SHORT REPORT

Two-colour FISH detection of the inv(16) in interphase nuclei of patients with acute myeloid leukaemia

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Summary. The inv(16)(p13q22) and t(16;16)(p13;q22) in acute myeloid leukaemia are associated with a relatively good prognosis but are difficult to detect using classic cytogenetics. We have designed a two-colour fluorescence *in situ* hybridization approach that uses two DNA probes that map close to and on either side of the inv(16) p-arm breakpoint region. This new strategy clearly detected the inv(16)(p13q22)/t(16;16)(p13;q22) on both metaphase chromosomes and in interphase nuclei, even when they are of poor quality. This procedure also detected the inv(16) in cases with an additional deletion of sequences proximal to the 16p-arm breakpoint which is present in 20% of all cases.

Keywords: AML, diagnosis, inv(16)(p13q22), t(16;16)(p13;q22), two-colour FISH.

The inv(16)(p13q22) and t(16;16)(p13;q22) are found in 10% of all cases with *de novo* acute myeloid leukaemia (AML, M4 Eo) (Le Beau *et al*, 1983). Because these rearrangements are recognized as positive prognostic factors, their detection is essential. The inv(16) is difficult to detect by classic cytogenetics but generates a CBFB-MYH11 fusion gene that can be detected by reverse transcriptase-polymerase chain reaction (Liu *et al*, 1993b). Although the latter method is generally favoured for inv(16) detection because of its superior sensitivity it is not impervious to error (Claxton *et al*, 1994; van der Reijden *et al*, 1997). Therefore additional inv(16) detection methods are desirable. We and others previously identified yeast artificial chromosomes (YACs) that span the 16p breakpoint (Dauwerse *et al*, 1993; Liu *et al*, 1993a). Initially, these YACs appeared to be excellent probes for interphase inv(16) detection in one-colour fluorescence *in situ* hybridization (FISH) showing three clearly separated signals: one from the unaffected chromosome, the other two from the disrupted YAC signal. However, an additional deletion of sequences proximal to the 16p-arm breakpoint, present in 20% of all inv(16) cases, causes the absence of the YAC signal proximal to the 16p-arm breakpoint (Dauwerse *et al*, 1993; Liu *et al*, 1993b; Marlton *et al*, 1995). The two resultant signals mimic the absence of an inv(16) and lead to false-negative results. The use of YACs as FISH probes for inv(16)/t(16;16) detection is therefore strongly discouraged. We describe a new sensitive two-colour FISH test for the detection of the inv(16) and t(16;16) in interphase nuclei.

MATERIALS AND METHODS

Patients and cytogenetics. Bone marrow or peripheral blood from inv(16)/t(16;16) patients and controls were obtained for cytogenetic analyses (Table I). Metaphases were obtained after culturing in fluorodeoxyuridine (FUDR) or methotrexate.

FISH. Cosmid probes were subcloned from YAC Y55.1 (Dauwerse *et al*, 1993). For FISH experiments (Dauwerse *et al*, 1992), cosmids were labelled separately by standard nick translation in the presence of biotin-11-dATP for the distal cosmids zit27, zit29 and zit80, or in the presence of digoxigenin-11-dUTP for the proximal cosmids zit14, zit18 and zit38.

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Table I. Distribution of two-colour FISH signals in interphase nuclei in inv(16)/t(16;16) and control samples according to the hybridization pattern of the proximal and distal probe sets.

Case	UPN	Karyotype	88 (%)	8oo (%)	8o (%)	8 (%)
1	B92-414	inv(16)	4·0	96·0	0·0	0·0
2*	B95-178	inv(16)	0·0	0·0	100·0	0·0
3	B92-619	inv(16)	2·0	94·0	4·0	0·0
4	H88-782	inv(16)	34·0	66·0	0·0	0·0
5	B88-423	inv(16)	5·0	95·0	0·0	0·0
6	H91-80	inv(16)	0·6	99·4	0·0	0·0
7	B95-658	t(16;16)	3·0	97·0	0·0	0·0
8*	B96-874	inv(16)	7·0	93·0	0·0	0·0
9*	B97-006CR	Normal	98·3	1·7	0·0	0·0
10	B96-111D	Normal	100·0	0·0	0·0	0·0
11	B96-110	Normal	98·3	1·7	0·0	0·0
12	B96-91	Normal	99·0	0·5	0·0	0·5
13	B96-76	−Y, t(8;13;21)	97·5	1·2	0·0	1·3
14	H96-60	Normal	99·3	0·0	0·0	0·7
15	B96-35D	Normal	98·7	1·3	0·0	0·0
16	B96-51	Normal	100·0	0·0	0·0	0·0
17	B95-875	Complex	100·0	0·0	0·0	0·0
18	ROS6	Normal	99·3	0·7	0·0	0·0
19	PHA BB	Normal	98·7	1·3	0·0	0·0
20	PHA BS	Normal	100·0	0·0	0·0	0·0

UPN = unique patient number. *Indicates new inv(16)/t(16;16) cases, other cases were reported previously (van der Reijden *et al*, 1995, 1996). All inv(16)/t(16;16) patients were classified as M4 Eo. For all cases 300 interphase nuclei were analysed, except for cases 12 and 13 (600 and 400 nuclei analysed). Case 9 is an inv(16) case in complete remission. Controls include donors (cases 10 and 15 [bone marrow] and 19 and 20 [blood cultures]) and AML/MDS patients without 16p aberrations (cases 11–14 and 16–17). Case 18 is a cell line with a normal karyotype (ROS6); %, frequency of signal distribution with: 88, two co-localizing signals; 8oo, one co-localizing signal and two separate signals; 8o, one co-localizing signal and one separate signal and 8, one co-localizing signal; Mean of false positives (% 8oo in controls 10–20) = 0·61% with standard deviation of 0·66. Cut-off value for minimal residual disease detection is mean + 3 times standard deviation = 2·6%.

RESULTS AND DISCUSSION

Firstly, two cosmid contigs near the 16p-arm breakpoint were defined. The cosmids zit14, zit18 and zit38 form the proximal contig (~100 kb), and the cosmids zit27, zit29 and zit80/(zit62) form the distal contig (~110 kb). Zit62 was initially used but showed cross-hybridization (not shown) and was therefore replaced by zit80 which does not cross-hybridize. The distance between the two contigs was determined to be 100–150 kb based on inv(16) YAC sizes (Dauwerse *et al*, 1992) and by using the cosmids as probes in fibre FISH experiments (not shown).

The FISH system was tested by hybridizing the two contigs in two separate colours to slides of a normal control case and three inv(16) patients. Two bright co-localizing red and green signals could be seen on both chromosomes 16 on metaphase spreads of the normal control (not shown). Likewise, bright co-localizing signals were observed in interphase nuclei (Fig 1A). On metaphase chromosomes of the three inv(16) patients, one double-colour signal on the normal chromosome 16 was seen, in addition to two separate signals on the inverted chromosome 16 (Fig 1B). Similarly, in interphase nuclei, one set of co-localizing signals of the unaffected chromosome 16 was observed in addition to two separated signals, reflecting the inverted chromosome 16 (Fig 1C).

To test the feasibility of the two-colour FISH approach, the probes were hybridized to slides of eight newly diagnosed cases [one t(16;16) and seven inv(16)] and one inv(16) case in complete remission (Table I). At least 300 interphase nuclei were analysed per case. Nuclei were not scored unless at least one set of red and green co-localizing signals from the normal chromosome 16 was observed. In all nuclei scored as harbouring an inv(16), the disruption of the 16p-arm locus was clearly demonstrated by the separation of the green and red signals. The percentage of normal nuclei at diagnosis varied between 0 and 34% (Table I). In the inv(16) case in complete remission, no inv(16) cells were detected.

The specificity of this system was determined by hybridizing the probes to metaphase/interphase spreads from 20

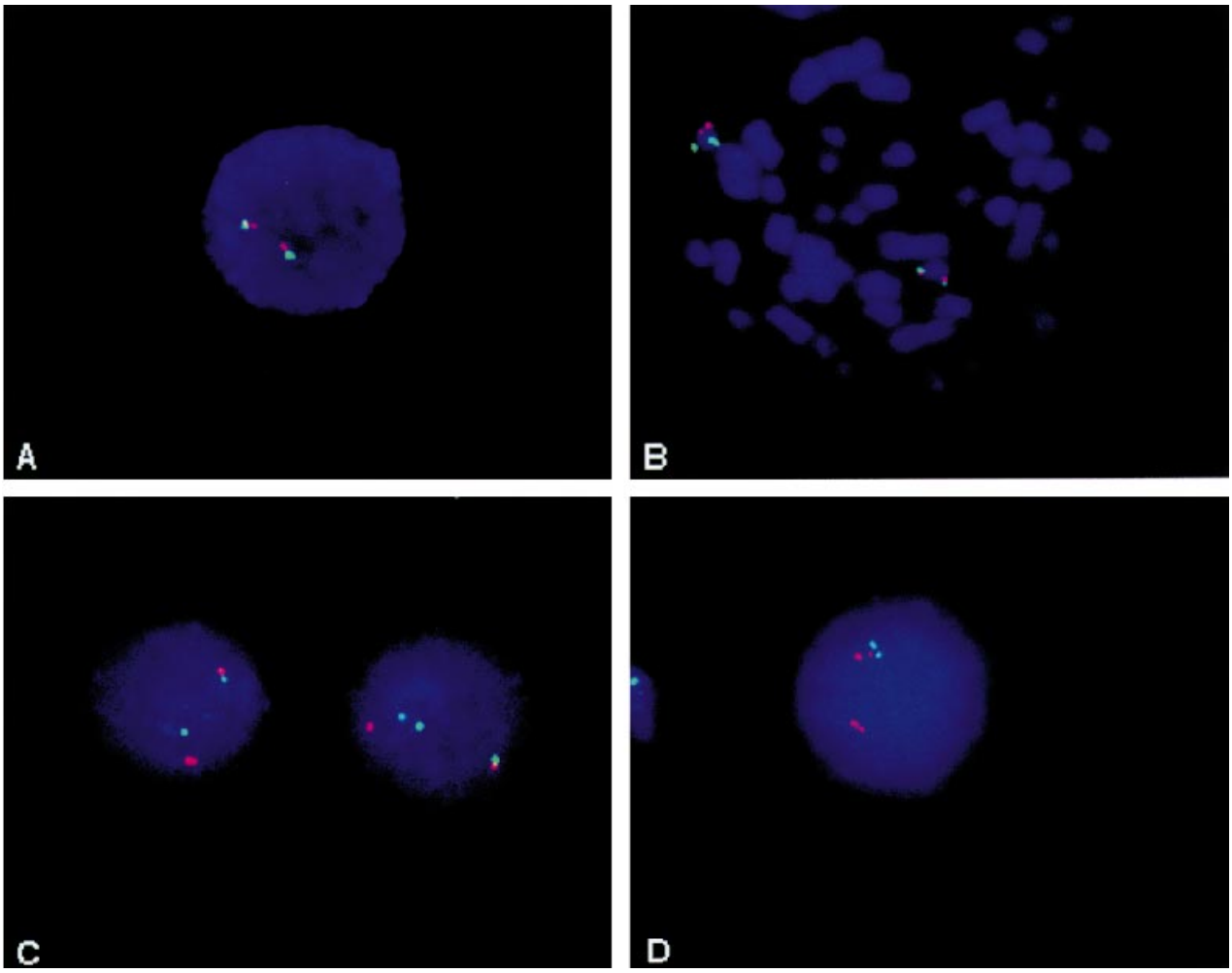

Fig 1. Hybridizations with the proximal (green) and distal (red) probe combination: (A) interphase nuclei of a normal control, (B) metaphase chromosomes of an inv(16) patient, (C) interphase nuclei of an inv(16) patient, (D) interphase nuclei of an inv(16) patient with an additional deletion of sequences proximal to the p-arm breakpoint. Single signals in the nuclei, depending on their phase in the cell cycle, can appear as two close hybridization signals (C and D).

controls (cases 10–20, Table I). At least 300 interphase nuclei were analysed per case. The cut-off value represents the technical limit for the detection of residual disease and was determined to be 2·6% (Table I). Therefore the FISH approach can detect minimal residual disease above this level.

Finally, an inv(16) patient with a known additional deletion of sequences proximal to the 16p breakpoint was tested. Co-localizing red and green signals on the normal chromosome 16 and only the red signal (distal contig) on the derivative 16p-arm of the inv(16) chromosome were observed in metaphase preparations (not shown). Likewise, the normal chromosome was represented by a double-colour red/green spot and the inverted chromosome 16 was represented by one red signal in interphase nuclei (Fig 1D). Data of a recent one-colour FISH study suggests that in 20% of inv(16) cases two distinct populations of cells are found; one with the deletion and one without (Martinet *et al*, 1997). With our more sensitive two-colour FISH approach we did not detect a subpopulation of cells with a deletion in the eight inv(16)/t(16;16) cases that were tested in detail, suggesting that such subpopulations do not exist.

We conclude that the two-colour FISH assay is very suitable for sensitive inv(16)/t(16;16) detection in interphase nuclei, even for cases that have the additional p-arm deletion.

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